Supplementary Material: Minimal amelogenin domain for enamel formation

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Supplemental Figure 1.
Strategy for the ADP7 CRISPR/CAS9 knock-in to the amelogenin gene locus on the X-chromosome (*Amelx*). Upper diagram shows the wild type *Amelx* which will be modified in the lower diagram to express ADP7 by knock-in to the amelogenin protein leader sequence in exon 2 to preserve protein secretion. Full-length amelogenin transcription is blocked by a TGA codon. The Msc1 restriction endonuclease site is created at the junction of the ADP7 and exon 2 to simplify identification of the knock-in versus the wild type allele. Restriction enzyme sites for Msc1 and resulting fragment sizes of 15.3 KB for wild type or 11.8 and 3.8 KB for ADP7 are shown. The orange line identifies intronic content while the blue boxes are exons; the thick horizontal blue lines identify the 5'- and 3'-hybridization probes. PCR primer positions are shown in pairs by thin blue arrowed lines and corresponding sequences are provided in Table 1.

Supplemental Figure 2.
Strategy for ADP7 knock-in to exon 2 of the mouse X-chromosome (*Amelx*) amelogenin. Panel A depicts the CRISPR sites within exon 2 of the mouse *Amelx* designed to retain in-frame position with the leader for protein secretion. Panel B shows the nucleic acid sequence for the guide RNAs and their production method.

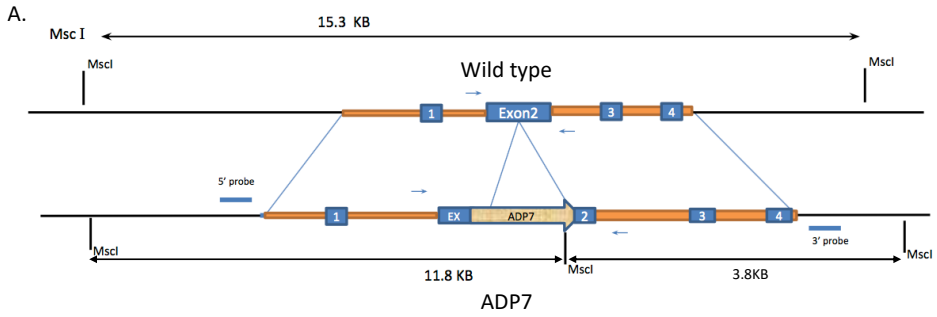
Supplemental Figure 3.
Southern blotting to identify the genotype of prodigy from founder lines 1153 and 1154. To distinguish between the wildtype (Wt) and the ADP7 knock-in, DNA was recovered and digested with restriction enzyme Msc1, hybridized to either a 5'- or 3'-probe to *Amelx* and the genotype confirmed by polymerase chain amplification. Panel A shows the 5'-probe detection of the Wt 15.3 kilo base pair (kbp) band or the 11.8 kbp band from the ADP7 knock-in. Panel B shows the 3'-probe detection of the Wt 15.3 kbp band or the 3.8 kbp band from the ADP7 knock-in. Panel C shows the size of the PCR amplicons confirming the genotype for the wildtype allele of 354 base pairs (bp) or the

ADP7 allele identified as a 497 bp band. Founder 1153 prodigy: 4523, male, Wt/Y; 4524 male, ADP7/Y; 4525 male, Wt/Y. Founder 1154 prodigy: 4678, female, ADP7/ADP7; 4679, female, ADP6/Wt; 4680 female, ADP7/Wt. DNA sequences for 5'- and 3'- probes and PCR primers are provided in Table 1.

Supplemental Figure 4.
Expression of ADP7 mRNA validated in postnatal day 3 incisors by reverse transcription followed by polymerase chain amplification. Incisors of 3-day old postnatal mice were recovered by dissection and total RNA was isolated. Reverse transcription was performed with an oligo dT primer and amplified with amelogenin exon 1 primer and ADP7 reverse primer. Panel A shows the amplicons grouped by the corresponding animal genotype. Panel B shows the nucleic acid sequence of the amplicon which corresponds to knock-in ADP7 allele. Homozygous mice express only mRNA corresponding to ADP7. Labels: Wt, wild type, ADP7, homozygous ADP7 knock-in minimal amelogenin.

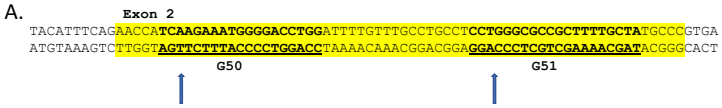
Supplemental Figure 5.
Histomorphometry and ameloblastin protein expression in incisor teeth. Panel A reveals normal histomorphometry of ameloblasts and extracellular matrix production for incisor teeth from wild type. Panel B is a Western blot for ameloblastin expression in incisors from 3-day-old littermates. Wildtype (Wt/Wt), heterozygous (ADP7/Wt) and homozygous (ADP7/ADP7) animals were used. Glycerol-3-phosphate dehydrogenase (GPDH) is provided as a loading control.

Supplemental Figure 1



Supplemental Figure 2.

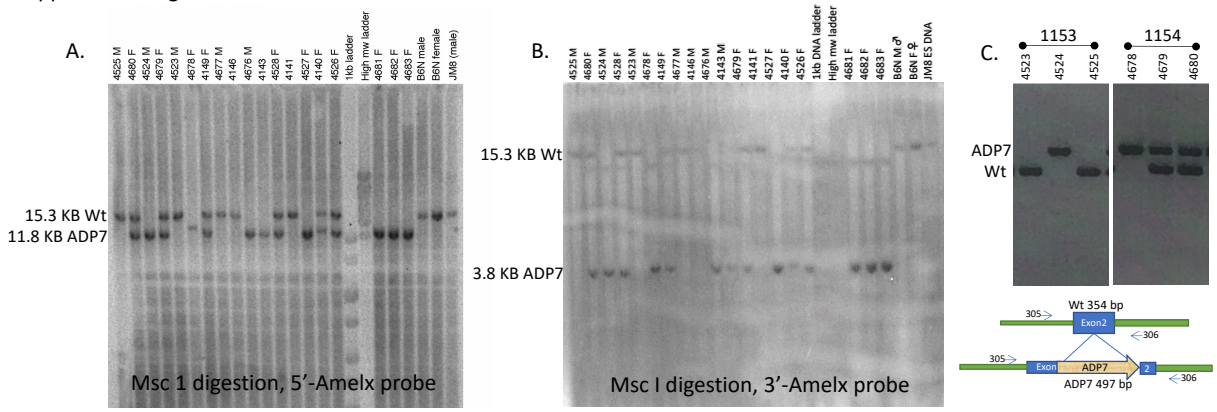
A.



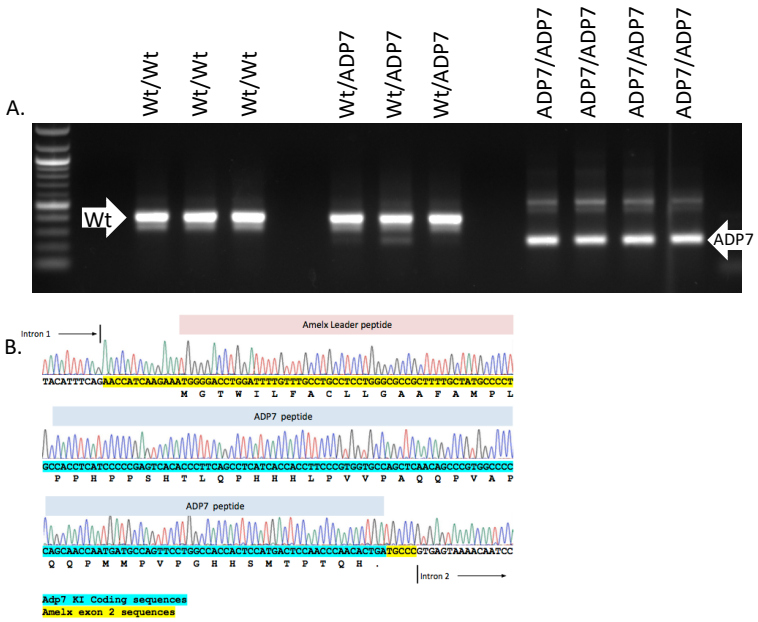

B.

| ID | Project | sequence | description | Strand | Template | production method |
|---|---|---|---|---|---|---|
| g50 | amlx | CCAGGTCCCCATTTCTTGA | 4 bp upstream ATG | Anti | SLQ1651 | 255/159 SLQ 1651 |
| g51 | amlx | TAGCAAAAGCTGCTCCCAGG | 22 bp upstream of 3' end Ex2 | Anti | SLQ1651 | 256/159 SLQ 1651 |

Supplemental Figure 3.



Supplemental Figure 4.




Supplemental Figure 5